

Microbial community profiles of the jejunum from steers differing in feed efficiency^{1,2,3}

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ABSTRACT: Research regarding the association between the microbial community and host feed efficiency in cattle has primarily focused on the rumen. However, the various microbial populations within the gastrointestinal tract as a whole are critical to the overall well-being of the host and need to be examined when determining the interplay between host and nonhost factors affecting feed efficiency. The objective of this study was to characterize the microbial communities of the jejunum among steers differing in feed efficiency. Within 2 contemporary groups of steers, individual ADFI and ADG were determined from animals fed the same diet. At the end of each feeding period, steers were ranked based on their standardized distance from the bivariate mean (ADG and ADFI). Four steers with the greatest deviation within each Cartesian quadrant were sampled ($n = 16/\text{group}$; 2 groups). Bacterial 16S rRNA gene amplicons were sequenced from the jejunum content using next-generation sequencing technology. The

phylum Firmicutes accounted for up to 90% of the populations within all samples and was dominated by the families Clostridiaceae and Ruminococcaceae. UniFrac principal coordinate analyses did not indicate any separation of microbial communities within the jejunum based on feed efficiency phenotype, and no significant changes were indicated by bacterial diversity or richness metrics. The relative abundances of microbial populations and operational taxonomic units did reveal significant differences between feed efficiency groups ($P < 0.05$), including the phylum Proteobacteria ($P = 0.030$); the families Lachnospiraceae ($P = 0.035$), Coriobacteriaceae ($P = 0.012$), and Sphingomonadaceae ($P = 0.035$); and the genera *Butyrivibrio* ($P = 0.019$), *Acidaminococcus* ($P = 0.018$), and *Ammoniphilus* ($P = 0.022$). The study identified jejunal microbial associations with feed efficiency, ADG, and ADFI. This study suggests the association of the jejunum microbial community as a factor influencing feed efficiency at the 16S level.

Key words: 16S ribosomal RNA, feed efficiency, jejunum, operational taxonomic units

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INTRODUCTION

Feed costs remain the largest variable input expense associated with beef production (Arthur et al., 2005). Additionally, because of rising feed costs, the increasing diversion of traditional livestock feedstuffs for production of biofuels is of growing concern (Galyean et al., 2011). Therefore, a better understanding of the microbial mechanisms associated with feed efficiency could lead to improved efficiencies as well as increase the profitability of livestock production enterprises.

Beef cattle host genetic contributions to feed efficiency have been difficult to pin down (Saatchi et al., 2014). This is likely due, in part, to differing genetics, feed regimens, and environments among studies. We

hypothesize that the microbial populations present along the cattle gastrointestinal tract (**GIT**) and variation among these populations may play important roles in determining the efficiency of breakdown and absorption of nutrients from feed. In ruminants, pregastric fermentation plays a key role in energy production and nutrient supply to the host, and therefore, much research has focused on the association between microbiota and feed efficiency in the rumen (Kim et al., 2011; Hernandez-Sanabria et al., 2012; McCann et al., 2014). Apart from recent research (Myer et al., 2015b,c), few other GIT communities have been examined in ruminants. Within the jejunum, important functions include the enzymatic breakdown of nutrients and their absorption. Accordingly, these jejunal/ruminal differences dictate their examination to fully understand the relationship between microbial populations along the beef cattle GIT and feed efficiency, ADG, and average daily DMI (ADFI).

To address this knowledge deficiency, we examined the microbial community of the jejunum from steers differing in feed efficiency using deep 16S rRNA-based community profiling. We hypothesize that variation in the microbial populations within the jejunum could contribute to variation in feed efficiency.

MATERIALS AND METHODS

Ethics Statement

This experiment was conducted to conform to the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010) and was approved by the U.S. Meat Animal Research Center Animal Care and Use Committee.

Experimental Design and Jejunum Content Sampling

Steers selected for this study came from a population of cattle being developed to have a high percentage of the following breeds: Angus, Beefmaster, Brahman, Brangus, Braunvieh, Charolais, Chiangus, Gelbvieh, Hereford, Limousin, Maine Anjou, Red Angus, Salers, Santa Gertrudis, Shorthorn, Simmental, South Devon, and Tarentaise. Each year heifers and cows were artificially inseminated with semen from prominent industry bulls of their dominant breed. This program resulted in offspring ranging from 50 to 75% of the same breed as their sire with the exception of Angus and Hereford, which ranged from 50 to 100% of the same breed as their sire. Individual feed intake was measured using an Insentec feeding system (Insentec B.V., Marknesse, The Netherlands). Steers were fed a ration (DM basis) of 57.35% dry-rolled corn, 30% wet distillers grain with solubles, 8% alfalfa hay, 4.25% supplement (containing

772 mg monensin/kg), and 0.4% urea. Feed intake and BW gain were measured over a 63-d period (Lindholm-Perry et al., 2013; Myer et al., 2015a). Steers were selected from 2 contemporary groups. Group 1 ($n = 148$) comprised spring-born calves that were 371 ± 1 d of age and weighed 522 ± 4 kg at the start of the feed intake measurement. Group 2 ($n = 197$) comprised fall-born calves that were 343 ± 1 d of age and weighed 448 ± 4 kg at the start of the feed intake measurement. At the end of each feeding period, ADFI and ADG were calculated. Subsequently, steers were ranked based on their standardized distance from the bivariate mean (ADG and ADFI) assuming a bivariate normal distribution with a calculated correlation between ADG and ADFI. Four steers with the greatest deviation within each Cartesian quadrant were sampled ($n = 16/\text{group}$; 2 groups). In the event a sire breed was overrepresented within a quadrant, a steer with the next highest rank of a different breed was selected. Quadrant 1 comprised steers that had greater ADG (2.14 ± 0.08 kg/d) and greater ADFI (12.76 ± 0.37 kg/d), quadrant 2 comprised steers that had greater ADG (1.84 ± 0.08 kg/d) and less ADFI (8.36 ± 0.08 kg/d), quadrant 3 comprised steers that had less ADG (1.26 ± 0.08 kg/d) and less ADFI (7.86 ± 0.08 kg/d), and quadrant 4 comprised steers that had less ADG (1.38 ± 0.08 kg/d) and greater ADFI (11.64 ± 0.08 kg/d). The result was a 2×2 factorial design consisting of greater and less ADFI and greater and less ADG (Myer et al., 2015a). Steers were allowed ad libitum access to feed within 1 h before harvest. At the end of the feeding period, steers were harvested and approximately 15 mL of jejunum contents were sampled. The 2 feeding studies yielded 32 animals for analysis. Immediately following sampling, samples were individually stored in buffered peptone water (pH 7.0) + 15% glycerol stock for processing and kept at -70°C for long-term storage after processing.

Deoxyribonucleic Acid Extraction, Amplification, and Sequencing

Deoxyribonucleic acid was extracted from jejunum samples using a repeated bead beating plus column method (Yu and Morrison, 2004). Briefly, 0.3 g of sample was centrifuged for 5 min at $16,000 \times g$ at 4°C to pellet solids including bacterial cells and then pelleted solids were resuspended in 0.2 mL Tris-EDTA (pH 8.0) buffer. Cell lysis was achieved by bead beating 0.15 g of the resuspended sample in ZR BashingBead Lysis Tubes (Zymo Research Corp., Santa Ana, CA) using the TissueLyser II system (Qiagen, Hilden, Germany) for 3 min at 21 Hz in the presence of 4% (wt/vol) SDS, 500 mM NaCl, and 50 mM EDTA. After mechanical and chemical cell lysis, 10 M ammonium acetate (260 μL) was used to precipitate and remove the impurities and

SDS followed by equal volume isopropanol precipitation for the recovery of the nucleic acids. Supernatants were treated with 2 μ L ribonuclease (10 mg/mL) and proteinase K (QIAamp DNA Stool Mini Kit) followed by the use of QIAamp columns from the Qiagen DNA Stool Mini Kit (Qiagen, Hilden, Germany). Genomic DNA concentration was determined using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE).

Amplicon library preparation was performed by PCR amplification of the V1 through V3 region of the 16S rRNA gene, using modified universal primers 27F (5'-adaptor/index/AGAGTTTGATCCTGGCTCAG) and 519R (5'-adaptor/index/GTATTACCGCGGCTGCTG) including TruSeq adapters sequences and indices as well as AccuPrime Taq high fidelity DNA Polymerase (Life Technologies, Carlsbad, CA). Amplification consisted of 23 cycles, with an annealing temperature of 58°C. Products were purified using AmPure bead purification (Agencourt AMPure, Beckman Coulter, Danvers, MA) and all libraries were quantified by the PicoGreen ds-DNA quantitation kit (Invitrogen Corp., Carlsbad, CA) and by real-time PCR on the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany). The PCR amplicon libraries were sequenced using the 2 \times 300, v3 600-cycle kit and the Illumina MiSeq sequencing platform (Illumina, Inc., San Diego, CA).

Sequence Read Processing and Analysis

All sequences were processed using the QIIME 1.8.0 software package (Caporaso et al., 2010). Paired reads were joined using fastq-join (Aronesty, 2011) and filtered for quality (sequences that had a mean quality score below Q25) using the Galaxy server (Blankenberg et al., 2010). Sequences that contained read lengths shorter than 400 bp were removed and adapters/index sequences were trimmed. Chimeric sequences were checked using ChimeraSlayer (Haas et al., 2011). All cleaned sequences were classified into taxa using the Greengenes 16S rRNA Gene Database (DeSantis et al., 2006). Operational taxonomic units (OTU) were calculated using the uclust program (0.03 dissimilarity; Edgar, 2010). After calculating richness for each quadrant, singletons were removed from further diversity analyses. Based on rarefaction curves, the number of OTU was normalized via subsampling 25,000 sequences from each jejunum sample. A phylogenetic tree was built with FastTree (Price et al., 2010) to determine α - and β -diversity metrics.

Statistical Analysis

All analyses were conducted using SAS 9.4 (SAS Inst. Inc., Cary, NC). The mean abundances ($n = 8$) of

data metrics and each taxon were compared among the feed efficiency groups using a model of contemporary group and Cartesian quadrant (greater ADG and greater ADFI [$\text{ADG}_{\text{Greater}} - \text{ADFI}_{\text{Greater}}$], greater ADG and less ADFI [$\text{ADG}_{\text{Greater}} - \text{ADFI}_{\text{Less}}$], less ADG and less ADFI [$\text{ADG}_{\text{Less}} - \text{ADFI}_{\text{Less}}$], and less ADG and greater ADFI [$\text{ADG}_{\text{Less}} - \text{ADFI}_{\text{Greater}}$]) as fixed effects. Significant differences were determined at $P < 0.05$ with the Benjamini–Hochberg method used for multiple-testing corrections (Benjamini and Hochberg, 1995). Multiple-testing corrections were made for the number of phyla, the number of OTU groups, and other classified taxa groups. Linear contrasts were then applied to significant quadrants to separate whether microbial populations varied by less vs. greater ADG, less vs. greater ADFI, or their interaction ($P < 0.05$). Principal coordinates analysis (PCoA) was performed using weighted and unweighted UniFrac analyses (Lozupone and Knight, 2005).

RESULTS

Diversity of Jejunum Bacterial Communities

The sampled jejunum contents of 32 steers, grouped into 4 feed efficiency phenotypes, resulted in a total of 10,847,155 sequence reads with an average read length of 500 bp after quality control and chimera detection and removal. Individual samples yielded an average of 338,974 cleaned sequence reads. Operational taxonomic units were defined as a bin of sequence reads sharing $\geq 97\%$ nucleotide sequence identity. Within the total cleaned sequences, a total of 32,663 OTU were detected with an average of $1,306 \pm 267$ per individual jejunal content sample. The average number of OTU detected from each Cartesian quadrant ranged from 1,134 to 1,455 OTU. Singletons accounted for approximately 48% of all OTU detected within the jejunum content samples. Coverage, as determined by Good's coverage estimator, ranged from 99.40 to 99.68%. Additionally, bacterial diversity (Shannon diversity index) ranged from 3.53 to 4.61.

Comparison among feed efficiency phenotype classes was conducted by normalizing the individual samples. Each sample OTU table was rarefied to 25,000 sequence reads, based on sample rarefaction curves, and singletons were then discarded. The normalized samples were then used for analysis using the sample means within each Cartesian quadrant. The normalized, cleaned sequences were examined using α -diversity metrics, consisting of bacterial diversity (Shannon diversity index), richness (Chao1), and coverage (Good's coverage estimator; Table 1). The overall OTU within each feed efficiency group did not differ significantly ($P > 0.05$), with an average of 499 ± 159 OTU per group. This was also paralleled by the

Table 1. Diversity statistics among sequences from grouped samples

Feed efficiency group ¹	Sampling type	No. of sequences	No. of OTU ^{2,3}	Chao1 ³	Shannon diversity index ³	Good's coverage, %
ADG _{Greater} -ADFI _{Greater}	Subsampled sequences ⁴	25,000	424 ± 109	588 ± 185	3.65 ± 1.22	99.59 ± 0.36
ADG _{Greater} -ADFI _{Less}	Subsampled sequences ⁴	25,000	504 ± 179	725 ± 224	4.61 ± 1.16	99.68 ± 0.25
ADG _{Less} -ADFI _{Less}	Subsampled sequences ⁴	25,000	534 ± 194	795 ± 198	3.85 ± 1.17	99.51 ± 0.19
ADG _{Less} -ADFI _{Greater}	Subsampled sequences ⁴	25,000	533 ± 145	895 ± 249	3.53 ± 1.09	99.40 ± 0.39

¹ADG_{Greater}-ADFI_{Greater} = greater ADG and greater ADFI; ADG_{Greater}-ADFI_{Less} = greater ADG and less ADFI; ADG_{Less}-ADFI_{Less} = less ADG and less ADFI; ADG_{Less}-ADFI_{Greater} = less ADG and greater ADFI. *n* = 8 among groups.

²OTU = operational taxonomic units.

³Within a column, means for the individual subsamples did not differ (*P* < 0.05).

⁴Means among the groups were compared using ANOVA and the Tukey's Test.

Chao1 richness metric, estimating 743 ± 195 OTU per group. Bacterial diversity indicated no significant differences among groups (*P* > 0.05), with a range of 3.53 to 4.61. Coverage was deemed adequate among feed efficiency phenotype groups, with a range of 99.40 to 99.68%, representative of the ADG_{Less}-ADFI_{Greater} and ADG_{Greater}-ADFI_{Less} groups, respectively.

Using an OTU-centric approach, the phylogeny-based method, UniFrac, was used to determine if the data separate into any sample clusters, via PCoA (Fig. 1). This β -diversity measure takes the phylogenetic divergence among the OTU into account when determining the differences within the jejunum microbial communities from each feed efficiency class (Lozupone et al., 2007). The PCoA of the jejunum microbial communities indicated no separation into

clusters in either the weighted (quantitative) and unweighted (qualitative) UniFrac distances of the jejunum microbial communities (Lozupone et al., 2011).

Taxonomic and Operational Taxonomic Unit Composition

The 10,847,155 cleaned sequences were classified using the Greengenes 16S rRNA Gene Database (DeSantis et al., 2006) resulting in 21 phyla, 51 classes, 94 orders, 198 families, and 397 genera. The unassigned taxa accounted for approximately 1.6% of the sequences. At the phylum level, Firmicutes dominated the data set across feed efficiency groups, ranging from 74 to 90% of the total sequences (Fig. 2a). This was similar to results demonstrated in previous studies regarding

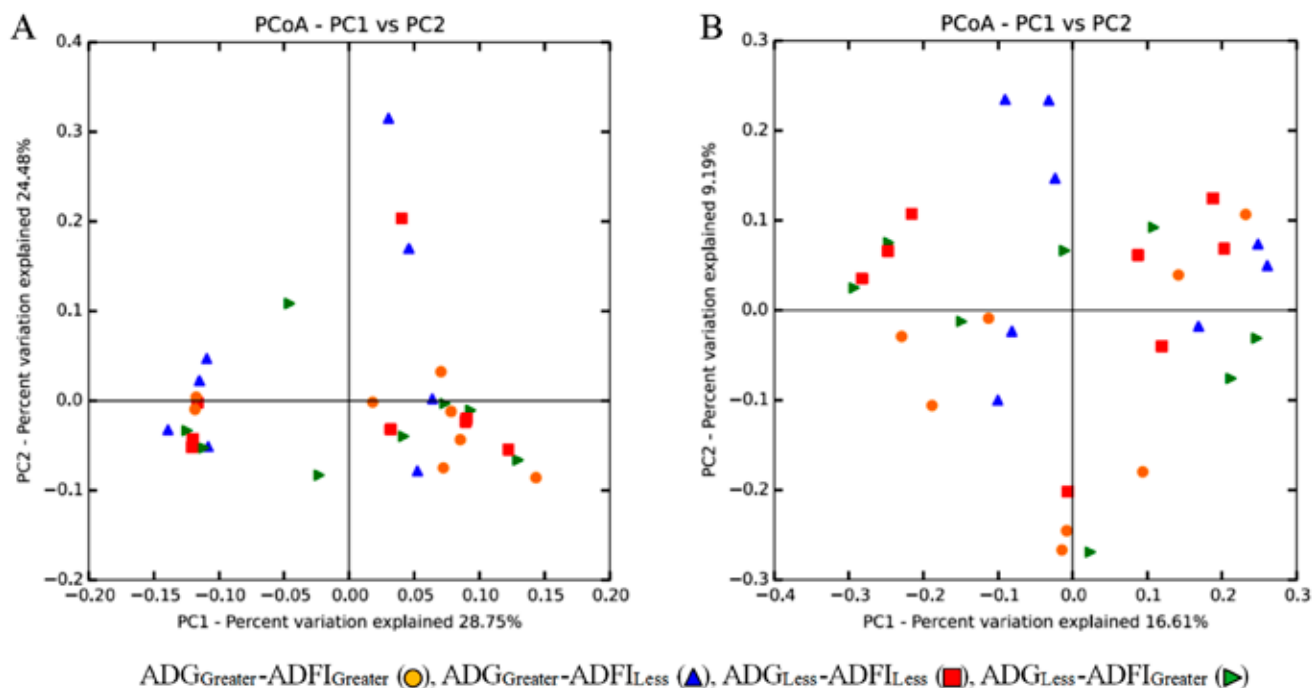


Figure 1. UniFrac (Lozupone and Knight, 2005) principal coordinates analysis (PCoA) displaying correlations among the bacterial communities of the 4 feed efficiency groups. A) Weighted PCoA analyzed from rarefied subsets of 25,000 sequences from each sample. B) Unweighted PCoA analyzed from rarefied subsets of 25,000 sequences from each sample. *n* = 8, represented by differing symbols. ADG_{Greater}-ADFI_{Greater} = greater ADG and greater ADFI; ADG_{Greater}-ADFI_{Less} = greater ADG and less ADFI; ADG_{Less}-ADFI_{Less} = less ADG and less ADFI; ADG_{Less}-ADFI_{Greater} = less ADG and greater ADFI.

microbial communities within the jejunum contents of cattle (de Oliveira et al., 2013; Malmuthuge and Griebel, 2014). Interestingly, the ADG_{Greater}–ADFI_{Less} group was noticeably reduced in the relative abundance of Firmicutes although not significantly ($P = 0.40$) relative to the remaining groups (74 to $90 \pm 6.97\%$). Additionally, the phylum Proteobacteria (0.8 to $5.8 \pm 1.28\%$) showed significant increases ($P = 0.030$) in relative abundance within the ADG_{Greater}–ADFI_{Less} group (Table 2). Other dominant phyla included Actinobacteria (6 to $13 \pm 5.65\%$), Tenericutes (0.4 to $4 \pm 1.58\%$), and Bacteroidetes (0.4 to $1.1 \pm 0.26\%$), but no significant differences between groups were observed (Fig. 2a). The remaining phyla accounted for less than 1% of the cleaned sequences and no significant differences were observed for these minor phyla across the groups.

At the genus level, *Ruminococcus* (12.2 to $19.6 \pm 3.64\%$), *Butyrivibrio* (2.6 to $7.7 \pm 1.30\%$), *Lactobacillus* (2.8 to $4.2 \pm 1.67\%$), *Bulleidia* (0.8 to $1.9 \pm 0.61\%$), *Mogibacterium* (1.1 to $1.7 \pm 0.28\%$), and *Mitsuokella* (0.05 to $1.27 \pm 0.46\%$) were detected at greatest relative abundance within the feed efficiency phenotype classes, and each represented $\geq 1\%$ of the total sequences (Fig. 2b) but did not differ among groups. *Propionibacterium* ranged from 0.07 to 7% (± 3.05) but no significant effect of group was observed. There were several taxa that were not classified to the genus level but were present in great relative abundance within the groups. Notably, these included the families Clostridiaceae (13.5 to $29.7 \pm 8.47\%$), Ruminococcaceae (13.9 to $19.6 \pm 3.64\%$), Micrococcaceae (0.1 to $8.9 \pm 4.61\%$), Lachnospiraceae (2.0 to $3.9 \pm 1.06\%$), and Coriobacteriaceae (1.5 to $4.3 \pm 1.72\%$) and additional classifications within the families Clostridiaceae (4.7 to $13.4 \pm 4.18\%$) and Lachnospiraceae (0.7 to $1.4 \pm 0.38\%$). Any remaining taxa were not listed and deemed nondetectable at relative abundances of $\leq 0.001\%$.

Several other taxa were identified at low relative abundances, but significant differences in relative abundance among feed efficiency groups were observed (Table 2). The genera *Acidaminococcus* ($P = 0.018$) and *Kaistobacter* ($P = 0.046$) were greatest in relative abundance within the ADG_{Greater}–ADFI_{Less} group, whereas *Ammoniphilus* ($P = 0.022$) and *Lysinibacillus* ($P = 0.041$) were most abundant within the ADG_{Less}–ADFI_{Greater} group. *Mycetocola* ($P = 0.031$) was least abundant within the ADG_{Greater}–ADFI_{Greater} group. Other taxa that were not classified to the genus level but demonstrated significant changes in relative abundance among the feed efficiency groups were the families Isosphaeraceae ($P = 0.039$) and Eubacteriaceae ($P = 0.049$), which were greatest in relative abundance within the ADG_{Less}–ADFI_{Greater} group, and Sphingomonadaceae ($P =$

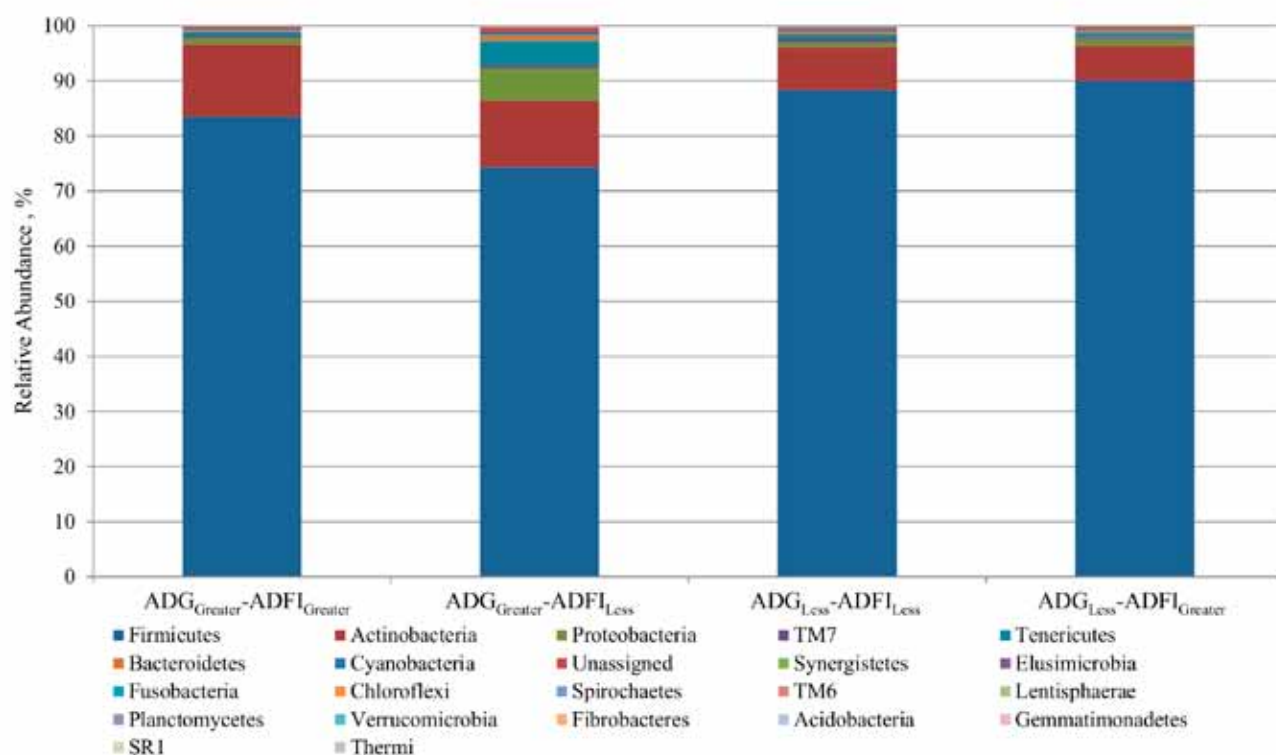
0.035), which were greatest in relative abundance within the ADG_{Greater}–ADFI_{Less} group. The family Planococcaceae ($P = 0.008$) was least abundant within the ADG_{Less}–ADFI_{Less} group. All taxa were defined as present in at least 50% of the samples.

The changes in relative abundance of OTU across all feed efficiency groups was also examined (Table 3). For this analysis, consideration was given to only OTU detectable at relative abundances $> 0.001\%$ and present in at least 50% of the samples. Several relative abundances within the genus *Butyrivibrio* were identified with significant differences in relative abundance among the groups, specifically increases within the ADG_{Greater}–ADFI_{Less} group. The OTU of greatest relative abundance that differed among the feed efficiency groups classified as *Butyrivibrio* and ranged from 1.9 to 6.0% (OTU-8445; $P = 0.041$). Four other OTU also classified as *Butyrivibrio*, and the lowest significant OTU ranged from 0.003 to 0.024% (OTU-3871; $P = 0.025$). The OTU classified as genus *Corynebacterium* (OTU-6355; $P = 0.029$) and the species *Lysinibacillus boronitolerans* (OTU-2387; $P = 0.040$) also demonstrated significant differences in relative abundance among feed efficiency groups, with greatest abundance within the ADG_{Greater}–ADFI_{Less} and ADG_{Less}–ADFI_{Greater} groups, respectively. Additionally, 3 OTU not classified to the genus level were different and included the families Mogibacteriaceae (OTU-4862; $P = 0.008$), Coriobacteriaceae (OTU-5116; $P = 0.012$), and Lachnospiraceae (OTU-5345; $P = 0.035$) and were greatest in relative abundance within the ADG_{Greater}–ADFI_{Less} group.

Effect of Gain and Intake

The effect of the microbial communities on ADG and ADFI additionally were analyzed to determine whether the associated microbial populations differed by less vs. greater ADG, less vs. greater ADFI, or their interaction. This was performed to examine the microbial population associations among the contributing factors of feed efficiency. The significant relative abundances of taxa and OTU between ADG and ADFI are listed in Tables 4 and 5, respectively. All significant taxa were either associated with ADG or associated with the combined ADG \times ADFI interaction. No taxa were associated with intake alone (Table 4), but 5 taxa were determined to have a significant effect for either gain or the interaction. When examined using OTU, 10 OTU were significant for either the main effect or the interaction. *Corynebacterium* (OTU-6355; $P = 0.004$) and the family Mogibacteriaceae (OTU-4862; $P = 0.011$) were significant for the interaction. Of the OTU identified as *Butyrivibrio*, 4 of the 5 were significant for ADFI (Table 5) and no other OTU was significant for this main effect. The remaining classified OTU

A



B

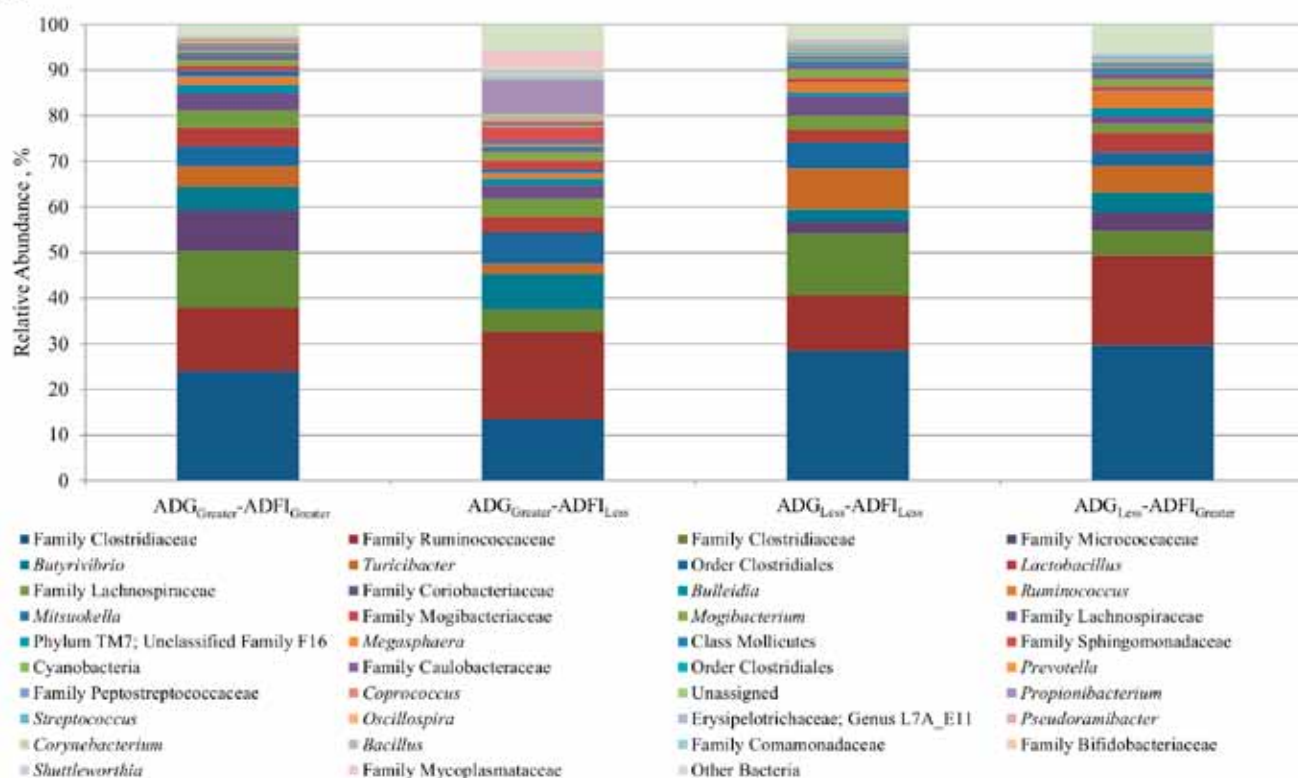


Figure 2. The taxonomic profiles for the relative phylum-level (A) and genus-level (B) abundance of each group classified by representation at $\geq 0.001\%$ of total sequences. Taxonomic composition of the jejunum microbiota among the 4 groups was compared based on the relative abundance (sequences of a taxon/total sequences in a sample). ADG_{Greater}-ADFI_{Greater} = greater ADG and greater ADFI; ADG_{Greater}-ADFI_{Less} = greater ADG and less ADFI; ADG_{Less}-ADFI_{Less} = less ADG and less ADFI; ADG_{Less}-ADFI_{Greater} = less ADG and greater ADFI.

Table 2. Relative abundance of significant taxa in the 4 feed efficiency groups

Classification	Percentage of total sequences ¹				SEM	P-value ²	No. of steers with detectable taxon ³
	ADG _{Greater} –ADFI _{Greater}	ADG _{Greater} –ADFI _{Less}	ADG _{Less} –ADFI _{Less}	ADG _{Less} –ADFI _{Greater}			
<i>Acidaminococcus</i>	0.003	0.023	0.004	0.000	0.005	0.018	20
<i>Ammoniphilus</i>	9.96×10^{-4}	4.98×10^{-4}	5.00×10^{-4}	3.50×10^{-3}	0.001	0.022	27
Family Eubacteriaceae	0.000	0.000	0.000	2.50×10^{-3}	0.001	0.049	19
Family Isosphaeraceae	0.000	0.000	0.000	0.003	0.001	0.039	18
Family Planococcaceae	0.011	0.001	6.78×10^{-19}	0.006	0.002	0.008	19
Family Sphingomonadaceae	0.317	2.977	0.137	0.203	0.760	0.035	20
<i>Kaistobacter</i>	5.03×10^{-4}	0.011	3.39×10^{-19}	3.39×10^{-19}	0.003	0.046	17
<i>Lysinibacillus</i>	0.009	0.008	0.013	0.027	0.005	0.041	30
<i>Mycetocola</i>	1.36×10^{-18}	0.001	0.002	0.009	0.002	0.031	17
Phylum Proteobacteria	1.186	5.873	0.909	1.194	1.285	0.030	32

¹ADG_{Greater}–ADFI_{Greater} = greater ADG and greater ADFI; ADG_{Greater}–ADFI_{Less} = greater ADG and less ADFI; ADG_{Less}–ADFI_{Less} = less ADG and less ADFI; ADG_{Less}–ADFI_{Greater} = less ADG and greater ADFI. Data is shown as least squares means ($n = 8/\text{group}$).

²Differences among the groups are significant at $P < 0.05$.

³The total number of steers was 32. All data are defined as taxa that are present in at least 50% of the samples.

with significant effects were associated with ADG, which included the families Coriobacteriaceae (OTU-5116; $P = 0.033$) and Lachnospiraceae (OTU-5345; $P = 0.024$) as well as the species *Lysinibacillus boronitolerans* (OTU-2387; $P = 0.032$).

DISCUSSION

Microbial–GIT associations are essential for the overall well-being of the animal host. In cattle, much study has appropriately focused on the microbiome of the rumen and its specialized role of supplying energy and protein to the animal. In recent years, rumen–microbial interactions have emphasized specifically feed efficiency, but this vision is limited, and with knowledge garnered from the GIT of other mammals it is imperative to examine the microbial effect on host nutrition and health throughout the entire GIT of cattle. The GIT has multiple regions with specific functions, and it has been demonstrated that there are differences among the microbial populations among the segments of the GIT (Frey et al., 2010; de Oliveira et al., 2013). Consequently, one cannot infer host–microbe associations farther upstream within the GIT from the sampling of digesta from sites more distal, for example, where fecal samples are used to examine the function of the entire GIT. The jejunum is important for host digestion of nutrients and absorption, and this study is among the first to assess the variation of microbial communities as a function of feed efficiency within the jejunum of steers.

Microbial abundance and diversity drastically decreases within the jejunum compared with other sections along the GIT (de Oliveira et al., 2013; Reti et al., 2013; Myer et al., 2015a,b,c). Previous ruminal studies have sampled to depths of $\leq 25,000$ sequences/

sample and have acquired adequate coverage (Jami and Mizrahi, 2012; Myer et al., 2015a). To characterize most of the bacterial OTU within the jejunum contents of the steer, the study normalized the samples to liberal depth of 25,000 sequences/sample. Depth estimates were also referenced from sample rarefaction curves. The study was able to recover approximately 99% of all OTU calculated at 0.03 dissimilarity, determined by Good's coverage estimator. Sequencing coverage estimates and rarefaction curves as well as previous coverage estimates all indicated adequate depth for jejunum microbial community analyses.

The use of next-generation sequencing technologies allowed for deeper sequencing of the GIT segment and greater identification of OTU when compared with previous studies (de Oliveira et al., 2013; Reti et al., 2013). However, next-generation sequencing is limited to shorter read lengths than traditionally generated by cloning and sequencing of full-length 16S rRNA genes. The 16S rRNA gene contains multiple regions containing variable sequences interspersed with conserved regions, of which the region spanning the V1 through V3 regions was selected for the present study. Despite the increased total number of observed OTU, diversity analyses across the 4 feed efficiency groups revealed no significant differences in the number of observed OTU, richness (Chao1), or diversity (Shannon diversity index). The close similarities in the community structures were also reflected in the weighted and unweighted UniFrac PCoA, of which structure relies on the phylogenetic divergence between the OTU. The microbial populations of the jejunum among feed efficiency phenotypes did not cluster by host phenotype, further supporting the similarities among the microbial communities within each group. It was anticipated that host specificity may

Table 3. Relative abundance of significant operational taxonomic units (OTU) in the 4 feed efficiency groups

OTU ID ¹	Classification	Percentage of total sequences ²				SEM	P-value ³	No. of steers with detectable taxon ⁴
		ADG _{Greater} –ADFI _{Greater}	ADG _{Greater} –ADFI _{Less}	ADG _{Less} –ADFI _{Less}	ADG _{Less} –ADFI _{Greater}			
denovo8457	<i>Butyrivibrio</i>	0.023	0.077	0.012	0.034	0.014	0.019	24
denovo3871	<i>Butyrivibrio</i>	0.004	0.024	0.003	0.010	0.005	0.025	26
denovo3098	<i>Butyrivibrio</i>	0.053	0.166	0.032	0.048	0.034	0.033	27
denovo5794	<i>Butyrivibrio</i>	0.025	0.066	0.011	0.030	0.013	0.034	27
denovo8445	<i>Butyrivibrio</i>	2.664	6.025	1.996	3.187	0.997	0.041	32
denovo6355	<i>Corynebacterium</i>	0.005	0.024	0.019	0.005	0.005	0.029	26
denovo5116	Family Coriobacteriaceae	0.012	0.045	0.010	0.009	0.008	0.012	25
denovo5345	Family Lachnospiraceae	0.025	0.066	0.017	0.002	0.015	0.035	26
denovo4862	Family Mogibacteriaceae	0.138	0.615	0.264	0.217	0.096	0.008	32
denovo2387	<i>Lysinibacillus boronitolerans</i>	0.009	0.009	0.013	0.027	0.005	0.040	25

¹ID = OTU identifier.

²ADG_{Greater}–ADFI_{Greater} = greater ADG and greater ADFI; ADG_{Greater}–ADFI_{Less} = greater ADG and less ADFI; ADG_{Less}–ADFI_{Less} = less ADG and less ADFI; ADG_{Less}–ADFI_{Greater} = less ADG and greater ADFI. Data is shown as least squares means ($n = 8/\text{group}$).

³Differences among the groups are significant at $P < 0.05$.

⁴The total number of steers was 32. Percentage of total sequences for steers with nondetectable OTU were treated as 0.001%, and all data are defined as OTU that are present in at least 50% of the samples.

play a role in the similarities observed within the jejunum microbial community diversity analyses, of which some degree of host specificity has been demonstrated in the rumen (Weimer et al., 2010). In the rumen, the association may be important because the rumen and its microbiota are predominantly responsible for the digestion of plant mass to fermentation products that the animal uses as nutrients. Accordingly, microbial community-level differences among feed efficiency groups in the rumen were not observed (Myer et al., 2015a). However, within the jejunum, the lack of differences observed among feed efficiency groups at the community level may simply indicate that the important variation in microbial communities lies at a finer resolution, such as differences in OTU and relative taxonomic abundances, where changes may not affect the phylogenetic diversity of the populations associated with the groups but specific changes in taxa and OTU may have profound functional effects. These finer changes may be expected within the jejunum, as similar variations were observed among feed efficiency groups within the cecum, colon, and rumen (Myer et al., 2015a,b,c).

The majority of the 16S rRNA gene sequences observed in all jejunum samples belonged to the phyla Firmicutes, Proteobacteria, Actinobacteria, Tenericutes, and Bacteroidetes, which have been shown to represent the majority of gut-associated phylotypes in a variety of mammals (Ley et al., 2008; Shanks et al., 2011). These associations suggest that the abundant phyla play critical roles in the microbial ecology of the mammalian gut. There were distinct observable differences at the phylum and subphylum levels, which were especially pronounced within the ADG_{Greater}–ADFI_{Less} group at both the phylum and genus levels. At the phylum level, shifts

in the relative proportions were apparent among feed efficiency groups for the phyla Firmicutes, Tenericutes, and Proteobacteria. A decreased prevalence of Firmicutes and increase of Tenericutes was observed within the ADG_{Greater}–ADFI_{Less} group, although only the shift in the relative abundance of Proteobacteria achieved statistical significance ($P < 0.030$). Interestingly, increases in the abundance of Firmicutes in the rumen have been associated with energy harvesting and correlated with increases of fat (Jami et al., 2014), but such a role in the lower GIT is unclear. The reported role and abundances of Firmicutes in the lower gut may also be confounded by the previous ambiguous assignment of the class Mollicutes, with reference to its place in Firmicutes or Tenericutes in sequences databases. Although considered to be part of the phylum Tenericutes (Ludwig et al., 2009), this controversial assignment may still be reported as a member of the Firmicutes, altering abundance estimates within the lower GIT (Turnbaugh et al., 2008). Nevertheless, the trending increase in the relative abundance of Tenericutes hints at associations with increased feed efficiency. Proteobacteria have also been demonstrated to negatively correlate with Firmicutes abundances (Cook et al., 1994) but also positively correlate with feed conversion ratio (Jami et al., 2014). It is apparent that these cross-phylum correlations necessitate further study to determine their overall correlations to feed efficiency.

The taxonomic levels below the phylum level were dominated by the families Clostridiaceae and Ruminococcaceae as well as the genera *Butyrivibrio* and *Lactobacillus*. This is in agreement with previous studies regarding the assessment of microbial communities across the GIT, in which members of the families Clostridiaceae and Ruminococcaceae dominated

Table 4. Relative abundance of significant taxa within ADG and ADFI phenotypes

Classification	Phenotype ¹				SEM	Effect	P-value ²
	ADG _{Greater} – ADFI _{Greater}	ADG _{Greater} – ADFI _{Less}	ADG _{Less} – ADFI _{Less}	ADG _{Less} – ADFI _{Greater}			
<i>Acidaminococcus</i>	0.0130	0.0023	0.0033	0.0120	0.004	Gain × intake	0.029
<i>Ammoniphilus</i>	0.0007	0.0020	0.0007	0.0020	0.001	Gain × intake	0.025
Family Planococcaceae	0.0061	0.0030	0.0053	0.0037	0.002	Gain × intake	0.002
<i>Lysinibacillus</i>	0.0088	0.0197	0.0108	0.0177	0.003	Gain	0.032
<i>Mycetocola</i>	0.0005	0.0055	0.0010	0.0050	0.002	Gain	0.030

¹ADG_{Greater}–ADFI_{Greater} = greater ADG and greater ADFI; ADG_{Greater}–ADFI_{Less} = greater ADG and less ADFI; ADG_{Less}–ADFI_{Less} = less ADG and less ADFI; ADG_{Less}–ADFI_{Greater} = less ADG and greater ADFI. Data is shown as least squares means ($n = 16/\text{phenotype}$).

²Differences among the groups are significant at $P < 0.05$.

the small intestine of a steer (de Oliveira et al., 2013). Additionally, similar profiles were identified in the jejunal digesta-associated bacterial communities in the GIT of calves (Malmuthuge and Griebel, 2014). Again, distinct observable differences were present within the ADG_{Greater}–ADFI_{Less} group.

There was little similarity between the rumen and the jejunum regarding the significant taxa and their relative abundance, which was anticipated due to changes in the functional tissue and environmental conditions within the rumen and jejunum (Myer et al., 2015a). This observation also aids the continuing support that the microbial populations within distal sections of the GIT are distinct from those of the rumen. Additionally, the microbial communities of the jejunum were distinct from those of the cecum and the colon (Myer et al., 2015b,c), demonstrating the microbial specificity of each niche and substantiating the functional association of each GIT section and its respective microbial community. However, overall the microbial communities of the cecum and colon were phylogenetically more similar to the jejunum than to that of the rumen (P.R. Myer, unpublished data), which also may be expected due to the great differences in functionality between the rumen and more distal sections of the GIT.

Significant differences in the relative abundance of specific taxa within the jejunum among the 4 groups were also observed. With reference to its putative functions, *Acidaminococcus* are AA-fermenting bacteria found in the rumen (Cook et al., 1994) and its presence in jejunal digesta of the most efficient animals may be indicative of greater GIT proteolytic activity for these animals. Interestingly, *Ammoniphilus* was observed, which is an obligately oxalotrophic, ammonium-dependent aerobic organism (Zaitsev et al., 1998). Oxalate degradation is common in the GIT and is preventative to the formation of kidney stones, but this activity has been associated with the anaerobic bacterium *Oxalobacter formigenes* in either the rumen or lower GIT (Allison et al., 1985). The role of the aerobic organism *Ammoniphilus* in the jejunum is unknown. Identification of significant differ-

ences among the family Sphingomonadaceae, which are α -proteobacteria, account for much of the observed phyla Proteobacteria. *Kaistobacter* is a genus within the family Sphingomonadaceae previously observed in soil communities (Ceja-Navarro et al., 2010), and although observed at low relative abundance, this genus shifted across the feed efficiency groups similar to Proteobacteria.

Examination of the relative abundance of significant OTU within the jejunum revealed many associations with the genus *Butyrivibrio*. The family Lachnospiraceae OTU may be related in function to the other significant *Butyrivibrio* OTU because *Butyrivibrio* is a member of the family Lachnospiraceae. Fiber digestion occurs not only in the rumen but also in the intestinal tract (Warner et al., 1972), and the hemicellulolytic activity of *Butyrivibrio*, combined with its abundance, plays an important role in fiber digestion. Yet the residence time in the jejunum may not be long enough to see significant fiber digestion. *Butyrivibrio* can ferment a wide range of sugars, which may explain its presence. Other significant OTU, such as the families Mogibacteriaceae and Coriobacteriaceae, are present in GIT microbial population analyses (de Menezes et al., 2011; Gharechahi et al., 2015), but little has been studied as to their functional association within the GIT of ruminants.

Feed efficiency is a function of gain and intake, and the potential effect of the components feed efficiency may be important. Significant identified taxa and OTU were examined to determine whether the microbial populations differed individually by gain (ADG) or intake (ADFI) or if an interaction was observed with the microbial group. Within both taxon and OTU analyses, the majority of the significantly identified taxa and OTU were associated with AGD or the interaction of ADG and ADFI. Interestingly, *Butyrivibrio* was the only assignment that was associated solely with intake. Research has demonstrated that in ruminants, animals with high performance, high energy density, and high ADFI tended to have decreased ruminal fiber digestibility (Tan et al., 2002). Digestible fiber that is not degraded in the rumen or escapes digestion in the

Table 5. Relative abundance of significant operational taxonomic units (OTU) within ADG and ADFI phenotypes

OTU ID ¹	Classification	Phenotype ²				SEM	Effect	P-value ³
		ADG _{Greater} – ADFI _{Greater}	ADG _{Greater} – ADFI _{Less}	ADG _{Less} – ADFI _{Less}	ADG _{Less} – ADFI _{Greater}			
denovo8457	<i>Butyrivibrio</i>	0.050	0.023	0.017	0.056	0.010	Intake	0.013
denovo3871	<i>Butyrivibrio</i>	0.014	0.006	0.003	0.017	0.004	Intake	0.014
denovo3098	<i>Butyrivibrio</i>	0.110	0.040	0.042	0.107	0.024	Gain	0.047
denovo5794	<i>Butyrivibrio</i>	0.046	0.021	0.018	0.048	0.009	Intake	0.031
denovo8445	<i>Butyrivibrio</i>	4.345	2.591	2.330	4.606	0.705	Intake	0.031
denovo6355	<i>Corynebacterium</i>	0.014	0.012	0.012	0.014	0.004	Gain × intake	0.004
denovo5116	Family Coriobacteriaceae	0.028	0.010	0.011	0.027	0.006	Gain	0.033
denovo5345	Family Lachnospiraceae	0.046	0.009	0.021	0.034	0.011	Gain	0.024
denovo4862	Family Mogibacteriaceae	0.376	0.240	0.201	0.416	0.068	Gain × intake	0.011
denovo2387	<i>Lysinibacillus boronitolerans</i>	0.009	0.020	0.011	0.018	0.003	Gain	0.032

¹ID = OTU identifier.

²ADG_{Greater}–ADFI_{Greater} = greater ADG and greater ADFI; ADG_{Greater}–ADFI_{Less} = greater ADG and less ADFI; ADG_{Less}–ADFI_{Less} = less ADG and less ADFI; ADG_{Less}–ADFI_{Greater} = less ADG and greater ADFI. Data is shown as least squares means ($n = 16$ /phenotype).

³Differences among the groups are significant at $P < 0.05$.

rumen becomes available for digestion in the lower GIT. Consequently, changes in the abundance of these organisms in the jejunum may play an important role in feed efficiency, which is supported by the data in this study. Again, residence time within the jejunum may limit the extent of cellulose digestion. Changes in the abundance of the butyrate-producing *Butyrivibrio* may influence the energy pool available to enterocytes, of which butyrate is known to be a primary metabolic fuel for enterocytes (Wächtershäuser and Stein, 2000; de Graaf et al., 2010). Among other effects butyrate has on gastrointestinal epithelial cells, such as epithelial cell proliferation and differentiation and colonic barrier function (Guilloteau et al., 2010), butyrate has been shown to influence leptin expression in bovine adipocytes, affecting the regulation of food intake and energy expenditure (Soliman et al., 2007; Arora et al., 2011). In summary, the known biology associated with *Butyrivibrio* and the GIT suggests that the observed shifts in prevalence could lead to significant changes in host feed efficiency.

Although likely a small percentage, it is important to note that some of the observed bacterial abundance may be a result of spillover from the rumen, either as surviving organisms or as residual DNA. However, common and abundant organisms in the rumen (Myer et al., 2015a), such as Ruminococcaceae, which exhibit cellulolytic activity in the rumen, are also important in the postruminal degradation of cellulose and starch in the small and large intestine. Moreover, given the short passage time through the jejunum, spillover organisms from the jejunum likely contribute to the presence and great abundance of these functional organisms within the distal GIT (Myer et al., 2015b,c). Yet the natural method of passage and residence of

these organisms should not overshadow their presence and function within respective GIT segments.

The majority of the taxa and OTU identified as associating with changes in feed efficiency in this study were related to the known fermentative and metabolic activities in the cattle jejunum, based on the putative microbial functions, and have been previously demonstrated as common components in jejunal microbial communities (de Oliveira et al., 2013). Although β -diversity metrics indicated no significant differences between the microbial communities of differing feed efficiency in steers, changes in the relative abundance of specific taxa and OTU were identified when the data were examined at higher resolution. These changes in the jejunum have been correlated with feed efficiency and may also play a role in affecting downstream associations with the digesta and microbial communities in the distal GIT. It must be noted, however, that although the changes in taxa and OTU and their putative functions in the jejunum can be correlated with the observed differences in the phenotypes, it is not clear whether changes in the microbiome are contributing to differences in feed efficiency or host factors are driving changes in the microbiome.

Previously, associations between the bovine ruminal microbiome and feed efficiency have been reported, but few studies have examined the relationship between the microbial communities in distal portions of the GIT and feed efficiency. Although the jejunum contains significantly fewer OTU and reduced phylogenetic diversity compared with the rumen (de Oliveira et al., 2013; Myer et al., 2015a), this study was able to identify specific associations with feed efficiency, ADG, and ADFI. However, as examination of farther distal portions of the GIT continues, studies may require greater sequencing depth to uncover changes in the abundances of taxa

and OTU as OTU count and diversity drastically increase. Additionally, the effect on feed efficiency may become less pronounced. Regardless, analyzing the microbial communities throughout the entire lower GIT should better aid in the complete understanding of the relationship between microbial populations in the GIT and feed efficient cattle.

LITERATURE CITED

- Allison, M. J., K. A. Dawson, W. R. Mayberry, and J. G. Foss. 1985. *Oxalobacter formigenes* gen. nov., sp. nov.: Oxalate-degrading anaerobes that inhabit the gastrointestinal tract. *Arch. Microbiol.* 141:1–7. doi:10.1007/BF00446731.
- Aronesty, E. 2011. ea-utils: Command-line tools for processing biological sequencing data. <http://code.google.com/p/ea-utils>. (Accessed 14 January 2014).
- Arora, T., R. Sharma, and G. Frost. 2011. Propionate. Anti-obesity and satiety enhancing factor? *Appetite* 56:511–551. doi:10.1016/j.appet.2011.01.016.
- Arthur, P. F., R. M. Herd, J. F. Wilkins, and J. A. Archer. 2005. Maternal productivity for Angus cows divergently selected for post-weaning residual feed intake. *Aust. J. Exp. Agric.* 45:985–993. doi:10.1071/EA05052.
- Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc., B* 57:289–300.
- Blankenberg, D., A. Gordon, G. Von Kuster, N. Coraor, J. Taylor, and A. Nekrutenko. 2010. Galaxy team. Manipulation of FASTQ data with Galaxy. *Bioinformatics* 26:1783–1785. doi:10.1093/bioinformatics/btq281.
- Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. Qiime allows analysis of high throughput community sequencing data. *Nat. Methods* 7:335–336. doi:10.1038/nmeth.f.303.
- Ceja-Navarro, J. A., F. N. Rivera-Orduna, L. Patino-Zúñiga, A. Vila-Sanjurjo, J. Crossa, B. Govaerts, and L. Dendooven. 2010. Phylogenetic and multivariate analyses to determine the effects of different tillage and residue management practices on soil bacterial communities. *Appl. Environ. Microbiol.* 76:3685–3691. doi:10.1128/AEM.02726-09.
- Cook, G. M., F. A. Rainey, G. Chen, E. Stackebrandt, and J. B. Russell. 1994. Emendation of the description of *Acidaminococcus fermentans*, a trans-aconitate- and citrate-oxidizing bacterium. *Int. J. Syst. Bacteriol.* 44:576–578. doi:10.1099/00207713-44-3-576.
- de Graaf, A. A., A. Maathuis, P. de Waard, N. E. P. Deutz, C. Dijkema, W. M. de Vos, and K. Venema. 2010. Profiling human gut bacterial metabolism and its kinetics using [U-13C] glucose and NMR. *NMR Biomed.* 23:2–12. doi:10.1002/nbm.1418.
- de Menezes, A. B., E. Lewis, M. O'Donovan, B. F. O'Neill, N. Clipson, and E. M. Doyle. 2011. Microbiome analysis of dairy cows fed pasture or total mixed ration diets. *FEMS Microbiol. Ecol.* 78:256–265. doi:10.1111/j.1574-6941.2011.01151.x.
- de Oliveira, M. N., K. A. Jewell, F. S. Freitas, L. A. Benjamin, M. R. Tótola, A. C. Borges, C. A. Moraes, and G. Suen. 2013. Characterizing the microbiota across the gastrointestinal tract of a Brazilian Nelore steer. *Vet. Microbiol.* 164:307–314. doi:10.1016/j.vetmic.2013.02.013.
- DeSantis, T. Z., P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, and G. L. Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* 72:5069–5072. doi:10.1128/AEM.03006-05.
- Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:2460–2461. doi:10.1093/bioinformatics/btq461.
- Federation of Animal Science Societies (FASS). 2010. Guide for the care and use of agricultural animals in agricultural research and teaching. 3rd. ed. FASS, Savoy, IL.
- Frey, J., A. Pell, R. Berthiaume, H. Lapierre, S. Lee, J. Ha, J. E. Mendell, and E. R. Angert. 2010. Comparative studies of microbial populations in the rumen, duodenum, ileum and faeces of lactating dairy cows. *J. Appl. Microbiol.* 108:1982–1993. doi:10.1111/j.1365-2672.2009.04602.x.
- Galyean, M. L., C. Ponce, and J. Schutz. 2011. The future of beef production in North America. *Anim. Front.* 1:29–36. doi:10.2527/af.2011-0013.
- Gharechahi, J., H. S. Zahiria, K. A. Noghabia, and G. H. Salekdeh. 2015. In-depth diversity analysis of the bacterial community resident in the camel rumen. *Syst. Appl. Microbiol.* 38:67–76. doi:10.1016/j.syapm.2014.09.004.
- Guilloteau, P. L., V. E. Martin, R. Ducatelle, R. Zabielski, and F. Van Immerseel. 2010. From the gut to the peripheral tissues: The multiple effects of butyrate. *Nutr. Res. Rev.* 23:366–384. doi:10.1017/S0954422410000247.
- Haas, B. J., D. Gevers, A. M. Earl, M. Feldgarden, D. V. Ward, G. Giannoukos, D. Ciulla, D. Tabbaa, S. K. Highlander, E. Sodergren, B. Methé, T. Z. DeSantis, The Human Microbiome Consortium, J. F. Petrosino, R. Knight, and B. W. Birren. 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res.* 21:494–504. doi:10.1101/gr.112730.110.
- Hernandez-Sanabria, E., L. A. Goonewardene, Z. Wang, O. N. Durunna, S. S. Moore, and L. L. Guan. 2012. Impact of feed efficiency and diet on adaptive variations in the bacterial community in the rumen fluid of cattle. *Appl. Environ. Microbiol.* 78:1203–1214. doi:10.1128/AEM.05114-11.
- Jami, E., and I. Mizrahi. 2012. Composition and similarity of bovine rumen microbiota across individual animals. *PLoS ONE* 7:e33306. doi:10.1371/journal.pone.0033306.
- Jami, E., B. A. White, and I. Mizrahi. 2014. Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. *PLoS ONE* 9:e85423. doi:10.1371/journal.pone.0085423.
- Kim, M., M. Morrison, and Z. Yu. 2011. Status of the phylogenetic diversity census of ruminal microbiomes. *FEMS Microbiol. Ecol.* 76:49–63. doi:10.1111/j.1574-6941.2010.01029.x.
- Ley, R. E., M. Hamady, C. Lozupone, P. J. Turnbaugh, R. R. Ramey, J. S. Bircher, M. L. Schlegel, T. A. Tucker, M. D. Schrenzel, R. Knight, and J. I. Gordon. 2008. Evolution of mammals and their gut microbes. *Science* 320:1647–1651. doi:10.1126/science.1155725.
- Lindholm-Perry, A. K., L. A. Kuehn, W. T. Oliver, A. K. Sexten, J. R. Miles, L. A. Rempel, R. A. Cushman, and H. C. Freetly. 2013. Adipose and muscle tissue gene expression of two genes (*NCAPG* and *LCORL*) located in a chromosomal region associated with cattle feed intake and gain. *PLoS ONE* 8:e80882. doi:10.1371/journal.pone.0080882.

- Lozupone, C. A., M. Hamady, S. T. Kelley, and R. Knight. 2007. Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. *Appl. Environ. Microbiol.* 73:1576–1585. doi:10.1128/AEM.01996-06.
- Lozupone, C. A., and R. Knight. 2005. Unifrac: A new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* 71:8228–8235. doi:10.1128/AEM.71.12.8228-8235.2005.
- Lozupone, C. A., M. E. Lladser, D. Knights, J. Stombaugh, and R. Knight. 2011. UniFrac: An effective distance metric for microbial community comparison. *ISME J.* 5:169–172. doi:10.1038/ismej.2010.133.
- Ludwig, W., K. H. Schleifer, and W. B. Whitman. 2009. Revised road map to the phylum Firmicutes. In: P. De Vos, G. M. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. A. Rainey, K.-H. Schleifer, and W. B. Whitman, editors, *Bergey's manual of systematic bacteriology*: Vol. 3: The Firmicutes. Springer New York. p. 1–13.
- Malmuthuge, N., and P. J. Griebel. 2014. Taxonomic identification of commensal bacteria associated with the mucosa and digesta throughout the gastrointestinal tracts of preweaned calves. *Appl. Environ. Microbiol.* 80:2021–2028. doi:10.1128/AEM.03864-13.
- McCann, J. C., L. M. Wiley, T. D. Forbes, F. M. Rouquette Jr., and L. O. Tedeschi. 2014. Relationship between the rumen microbiome and residual feed intake efficiency of Brahman bulls stocked on bermudagrass pastures. *PLoS ONE* 9:e91864. doi:10.1371/journal.pone.0091864.
- Myer, P. R., T. P. L. Smith, J. E. Wells, L. A. Kuehn, and H. C. Freetly. 2015a. Rumen microbiome from steers differing in feed efficiency. *PLoS ONE* 10:e0129174. doi:10.1371/journal.pone.0129174.
- Myer, P. R., J. E. Wells, T. P. L. Smith, L. A. Kuehn, and H. C. Freetly. 2015b. Cecum microbial communities from steers differing in feed efficiency. *J. Anim. Sci.* 93:5327–5340. doi:10.2527/jas.2015-9415.
- Myer, P. R., J. E. Wells, T. P. L. Smith, L. A. Kuehn, and H. C. Freetly. 2015c. Microbial community profiles of the colon from steers differing in feed efficiency. *SpringerPlus* 4(1):1–13. doi:10.1186/s40064-015-1201-6.
- Price, M. N., P. S. Dehal, and A. P. Arkin. 2010. FastTree 2 – Approximately maximum-likelihood trees for large alignments. *PLoS ONE* 5:e9490. doi:10.1371/journal.pone.0009490.
- Reti, K. L., M. C. Thomas, L. J. Yanke, L. B. Selinger, and G. D. Inglis. 2013. Effect of antimicrobial growth promoter administration on the intestinal microbiota of beef cattle. *Gut Pathog.* 5:8. doi:10.1186/1757-4749-5-8.
- Saatchi, M., J. E. Beever, J. E. Decker, D. B. Faulkner, H. C. Freetly, S. L. Hansen, H. Yampara-Iquise, K. A. Johnson, S. D. Kachman, M. S. Kerley, J. Kim, D. D. Loy, E. Marques, H. L. Neiberger, E. J. Pollak, R. D. Schnabel, C. M. Seabury, D. W. Shike, W. M. Snelling, M. L. Spangler, R. L. Weaver, D. J. Garrick, and J. F. Taylor. 2014. QTLs associated with dry matter intake, metabolic mid-test weight, growth and feed efficiency have little overlap across 4 beef cattle studies. *BMC Genomics* 15:1004. doi:10.1186/1471-2164-15-1004.
- Shanks, O. C., C. A. Kelty, S. Archibeque, M. Jenkins, R. J. Newton, S. L. McLellan, S. M. Huse, and M. L. Sogin. 2011. Community structures of fecal bacteria in cattle from different animal feeding operations. *Appl. Environ. Microbiol.* 77:2992–3001. doi:10.1128/AEM.02988-10.
- Soliman, M., K. Kimura, M. Ahmed, D. Yamaji, Y. Matsushita, Y. Okamatsu-Ogura, K. Makondo, and M. Saito. 2007. Inverse regulation of leptin mRNA expression by short- and long-chain fatty acids in cultured bovine adipocytes. *Domest. Anim. Endocrinol.* 33:400–409. doi:10.1016/j.domaniend.2006.08.005.
- Tan, Z. L., D. X. Lu, M. Hu, W. Y. Niu, C. Y. Han, and X. P. Ren. 2002. Effect of dietary structural to nonstructural carbohydrate ratio on rumen degradability and digestibility of fiber fractions of wheat straw in sheep. *Asian-Australas. J. Anim. Sci.* 15:1591–1598. doi:10.5713/ajas.2002.1591.
- Turnbaugh, P. J., F. Bäckhed, L. Fulton, and J. I. Gordon. 2008. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 3:213–223. doi:10.1016/j.chom.2008.02.015.
- Wächtershäuser, A., and J. Stein. 2000. Rationale for the luminal provision of butyrate in intestinal diseases. *Eur. J. Nutr.* 39:164–171. doi:10.1007/s003940070020.
- Warner, R. L., G. E. Mitchell, and C. O. Little. 1972. Post-ruminal digestion of cellulose in wethers and steers. *J. Anim. Sci.* 34:161–165.
- Weimer, P. J., D. M. Stevenson, H. C. Mantovani, and S. L. C. Man. 2010. Host specificity of the ruminal bacterial community in the dairy cow following near-total exchange of ruminal contents. *J. Dairy Sci.* 93:5902–5912. doi:10.3168/jds.2010-3500.
- Yu, Z., and M. Morrison. 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Biotechniques* 36:808–813.
- Zaitsev, G. M., I. V. Tsitko, F. A. Rainey, Y. A. Trotsenko, J. S. Uotila, E. Stackebrandt, and M. S. Salkinoja-Salonen. 1998. New aerobic ammonium-dependent obligately oxalotrophic bacteria: Description of *Ammoniphilus oxalaticus* gen. nov., sp. nov. and *Ammoniphilus oxalivorans* gen. nov., sp. nov. *Int. J. Syst. Bacteriol.* 48:151–163. doi:10.1099/00207713-48-1-151.